THE ACTION OF PROTOVERATRINE AND ACONITINE ON THE NEURO-MUSCULAR APPARATUS OF THE FROG.

By Marjory Macnaughton. (From the Physiological Laboratory, Edinburgh University.)

Gunning Prize in

Physiology.

1913

INTRODUCTION.

The substance protoveratrine was originally isolated from Veratrum album by Salzberger (1890) in Boehm's laboratory in Leipzig. Its pharmacological action was the subject of a communication by Watts Eden (1). He concluded that in the frog protoveratrine acts upon the medulla oblongata, the spinal cord and sensory nerve endings, and on skeletal muscle. He pointed out that a nerve muscle preparation taken from a frog which had been previously injected with protoveratrine shews a rapid and permanent fatigue when the nerve is repeatedly stimulated. He concluded that the protoveratrine acts chiefly upon the muscle fibres themselves.

Waller (2) next tested the action of protoveratrine both on nerve and on muscle. He found that in a concentration in which it has little or no effect upon muscle it exercises a very decided effect upon medullated nerve fibres - an effect comparable to fatigue. Its action on nerve he investigated solely with the galvanometer. Nerves were dissected from frogs that had been poisoned with the alkaloid. These were "tetanised" for short periods at regular intervals and the galvanometer swing photographically recorded. A pronounced negative variation occurred on the first stimulation of the nerve. This was followed, however, by no positive after-effect on cessation of the stimulation. The following periods of stimulation shewed less and less negative variation until the nerve soon ceased to give any electrical response. In no case did he observe a return swing of the galvanometer or positive after-effect. He

compared this effect of protoveratrine upon nerve with the well-known effect of veratrine upon muscle, and summed up the result thus :-

> "Veratrine acts upon muscle: veratrine does not act upon nerve. Protoveratrine acts upon nerve: protoveratrine does not act upon muscle."

Aconitine, he found, gives a similar result to protoveratrine.

Both Watts Eden and Waller obtained their supply of protoveratrine from Boehm, under whose direction Salzberger had originally isolated it. The substance has never been put on the market.

Tait (3), comparing the literature on protoveratrine with that relating to another alkaloid yohimbine, concluded that the two substances are almost identical in their pharmacological action. He procured a new supply of protoveratrine and Waller (4) tested this material as well as yohimbine by the galvanometer method. He found that the alkaloids tested side by side show quite different reactions - a positive after-effect being obtained with yohimbine in every case.

Wedensky (5) some years ago introduced a new and sensitive method of testing the action of a drug on nerve, the muscle response being used as an index of the processes occurring in the nerve. This method consists in applying to the proximal end of the nerve a series of very rapid rythmical stimulations, - a more distal part of the nerve having been previously subjected to the action of the substance which it is desired to investigate. Under these conditions the muscle response, instead of being a

regular tetanus, becomes modified in various ways, according to the rapidity and strength of stimulation. This was the method by which Tait and Gunn (6) had investigated yohimbine. Seeing that Waller had published no results shewing the modification in the muscle response when the nerve has been subjected to the action of protoveratrine or aconitine, it seemed worth while to investigate the action of protoveratrine and aconitine on nerve using Wedensky's method.

This investigation I undertook at the suggestion of Dr. Tait, to whom I am indebted for my supply of protoveratrine.

TECHNIQUE.

I. Frogs.

The frogs used were mostly Rana temporaria - a few Rana esculenta. As Watts Eden states, there is no difference between the two species, nor between male and female frogs with regard to their reaction to protoveratrine.

The frogs used for experiments had their brains destroyed, except for a few which Dr. Tait injected for me.

Experiments were carried out in June and July and in October and November.

II. Methods of subjecting the nerve to the action of the Protoveratrine.

The following methods were used :-

(1) Injection of the animal as a whole.

(2) Direct application of a solution of protoveratrine to the dissected nerve.

(3) Perfusion of the animal through the blood vessels.

(4) Soaking the whole nerve-muscle preparation, with the exception of the proximal portion of the nerve, in a solution of protoveratrine.

(1) Injection.

This was the method used by Waller.

A small quantity of protoveratrine (.02 - .04 mgs. per grm. wt. of frog), dissolved in 1 - 2 cc's of Ringer's solution, was injected into the dorsal lymph sac. By pinching up the portion of skin punctured by the needle, passing a fine

pin through it, and tying a ligature tightly under the pin, any escape of the solution through the puncture was prevented. The spinal cord of the frog was pithed after a certain length of time (in most cases after one hour). The sciatic-gastrocnemius preparation was then dissected out and enclosed in a moist chamber.

A number of experiments were also carried out in which the muscle and myo-neural junction were excluded from the action of the alkaloid by ligature of the thigh as in Claude Bernard's curari experiment. The portion of nerve outside the ligature was covered with a small piece of cotton wool soaked in Ringer's solution to keep it moist.

(2) Direct application to the nerve.—

The method was the one used by Tait and Gunn in their investigation of yohimbine.

The protoveratrine may be directly applied to the nerve of a dissected preparation either by enclosing the nerve between two narrow strips of blotting paper soaked in a solution of the alkaloid, or by allowing a loop of the nerve to dip into a small watch glass containing the solution. In either case the protoveratrine is applied only to the middle portion of the nerve, the part in contact with the electrodes remaining unaffected. By this method the action of the drug, in modifying simply the conductivity of the nerve, can be investigated.

The strength of solution used was 1 mg. of protoveratrine in 2 cc'ss of Ringer's solution. In this con-

concentration it took from two to three hours before the protoveratrine showed any signs of affecting the nerve.

The preparation was, of course, enclosed in a moist chamber.

(3) Perfusion.

Experiments were also carried out in which the whole animal was perfused through the aorta with a solution of protoveratrine. The bloodvessels were first washed out with Ringer's fluid and then perfused with a 1 in 10,000 solution of protoveratrine. The perfusion lasted for thirty minutes. Several experiments were performed in which the muscle and myo-neural junction were protected as in Claude Bernard's experiment.

(4) Soaking the muscle and distal portion of the nerve.

Dissected nerve muscle preparations were arranged in a bath containing a 1 in 10,000 solution of protoveratrine in such a way that, while the muscle and distal portion of the nerve were immersed in the solution, the proximal portion of the nerve (to which the stimulating electrodes were applied) was supported above the level of the liquid, being at the same time protected from evaporation.

III. Protoveratrine: Solution and Dose.

Solution. The protoveratrine was dissolved in Ringer's solution with the aid of a trace of lactic acid. Control experiments were carried out which showed that the lactic acid, in the concentration used, had absolutely no effect

upon nerve or muscle.

Dose. The amount of the dose, in those experiments in which the animal is injected, is an important factor in determining the nature of the result. If a dose is given so large that it kills rapidly, the preparation after dissection is found to be entirely unexcitable through the nerve; the muscle also is injuriously affected by the drug. With too small a dose the nerve is scarcely affected. Constant results cannot be obtained by simply injecting a small quantity of protoveratrine and waiting until the animal is completely paralysed:- a definite dose per gram weight must be given and the preparation dissected after a definite time has elapsed. The inconstant nature of Watts Eden's results is due to his neglect of these precautions.

It is of interest to find that the dose required varies with the state of nutrition of the animal. I found that with summer frogs a dose of .02 mgs. of protoveratrine per grm. wt. of frog (this is more than a lethal dose) would bring the nerve in the space of one hour into a suitable condition for experiment. I had noticed that the nerve-muscle preparation from some freshly caught frogs which I obtained in July were rather more resistant to the action of the protoveratrine than those that had beem kept in the laboratory all summer; but on resuming my experiments in October, I was surprised to find that a dose of .04 mgs. per grm. wt. was required before the desired

result could be obtained. This result cannot be explained by the fact that the poison takes effect much more slowly at low temperature. The laboratory temperature varies very little.

It is thus evident that the nerve (nerve fibres or myo-neural junction, or both) is more resistant to the action of the alkaloid in a well fed than in a starved animal.

IV. Stimulating Coil.

A standard Kronecker coil was used, in the primary circuit of which was placed an accumulator of two volts kept fully charged. The nerve was stimulated at a rate of either 25 or 100 induction shocks per second. The intensity varied between 30 and 200 Kronecker units - a strength at which physical spread of the current could not occur.

ACONITINE.

The action of Aconitine was examined in the same way as that of protoveratrine. Both the English and German preparation of aconitine was tested : no difference was observed in their action.

Injection.

A. General Results.

The general symptoms produced by the injection of protoveratrine have been described by Watts Eden and need only be briefly mentioned here. Respiration becomes slowed, and there are frequent gaping movements; there is increasing muscular weakness with rapid fatigue following activity; convulsions occur at intervals, which leave the animal absolutely exhausted and irresponsive to stimulation for some time after each attack: the recovery from these convulsions becomes less and less perfect, the frog's movements getting gradually feebler until a general paralysis ensues, which is followed by death. The heart is only slowly affected, and continues to beat for a considerable time after the skeletal muscles are paralysed.

Some points not recorded by Watts Eden may be noted. The lymph-hearts, which depend for their activity on nerve impulses received from the spinal cord, and in the wall of which striped muscle fibres are found, share in the general muscular paralysis and cease to beat at a very early period. The gaping may be produced immediately by spraying a solution of protoveratrine into the frog's mouth. It is probably caused, therefore, by a local irritation, and not by a direct action on the central nervous system, as Watts Eden suggested. The fact that the mucous membranes in an injected animal become engorged and secrete mucus freely is an additional argument in

favour of this view, and Watts Eden himself notes that the powdered plant causes sneezing when applied to the nasal mucous membrane. No "snap reflex", such as Gunn (7) described in yohimbinised frogs, is to be obtained from a frog under the influence of protoveratrine.

B. The Nerve Muscle Preparation.

When the sciatic-gastrocnemius preparation from a frog injected with a suitable dose of protoveratrine is tested by subjecting the nerve to short periods of rapid rythmical stimulation, alternating with short periods of rest, the result is similar to that shown in fig. 1. The muscle responds with perhaps one full tetanus, then with a series of broken tetani which rapidly decrease in height, then with a short series of single twitches which fall off in height until no further response can be elicited. At the beginning of such an experiment the preparation may show some slight signs of recovery after rest (fig. 2), but as it becomes more and more fatigued, rest ceases to have any restorative effect - indeed there is sometimes a distinct falling off during a period of rest (fig. 1). When the preparation has ceased to respond to a low intensity of stimulation (30 Kronecker units) it will not respond to a higher intensity (100 Kronecker units). After the muscle has once ceased to respond to excitation applied to the nerve, a rest of from one to thirty minutes will not restore excitability to indirect stimulation, nor will soaking for twelve hours in Ringer's solution. The

muscle, however, remains perfectly excitable to direct stimulation and shows no undue fatigue.

The result cannot be ascribed merely to a depression of excitability in the portion of the nerve stimulated. For one thing the failure of the preparation to respond to an increase in the strength of the stimulus shows that such is not the case. Nor can any response be obtained by shifting the electrodes so as to stimulate a fresh portion of the nerve. We are evidently dealing with a depression of conductivity in the neural elements of the preparation. That this depression of conductivity is not due simply to progressive and rapid invasion of the nervous structures by the protoveratrine can be shown in two ways. (1) Both preparations are dissected out; one is tetanized once; the other is then exhausted by repeated stimulation; on testing the first again it will be found to be still excitable through the nerve. Or (2) one preparation may be exhausted by repeatedly stimulating the pelvic plexus on that side before dissection: if both preparations are now dissected out, the one which was previously stimulated will be found to be unexcitable through the nerve, while the other gives the usual tracing. It is thus evident that activity is the determining factor in producing the rapid fall in conductivity in the nerve - in other words, that we are dealing with a fatigue phenomenon. This fatigue, unlike that obtained in any other experiments on nerve, is of a permanent nature.

If too small a dose of protoveratrine has been injected, or if the preparation has been dissected out too soon, the fatigue is not so rapid and the preparation shows a slight power of recovery after rest (fig. 2). If, on the other hand, too large a dose of the alkaloid has been given, or if the preparation has been left too long before dissection, it will be found to be unexcitable through the nerve. Watts Eden states that if the muscle cannot be stimulated through the nerve it will not respond to direct stimulation. This is certainly not the case. The muscle remains responsive to direct stimulation for many hours after stimulation through the nerve has become ineffective.

The result, then, of testing by the Wedensky method a nerve muscle preparation from a frog injected with protoveratrine is to produce a rapid and permanent loss of conductivity in the neural elements. At first sight it would seem easy to correlate this result with that obtained electrically by Waller when the nerve alone was subjected to experiment.

C. Direct Application.

This permanent fatigue, however, is not obtained when the protoveratrine is applied directly to the nerve of a dissected preparation.

The protoveratrine solution is applied, as already described, to the middle portion of the nerve. The preparation is tested at intervals of ten or fifteen minutes. After about two hours the muscular response begins to fall off for low intensities and rapid rates of stimulation, and this

process continues until the conductivity of the nerve is completely abolished. If, when the nerve is beginning to show signs of being affected by the protoveratrine, it is removed from the solution to prevent absorption of more poison and subjected to successive short periods of stimulation, it shows a progressive falling off in conductivity until it may at last cease to conduct ~~(fig. 4)~~. This is not a fatigue of the muscle: full tetani can be obtained by stimulating the distal portion of the nerve. So far the result resembles that obtained from a preparation taken from an injected frog. The loss of conductivity, however, though it may be complete, is not permanent. After a sufficient period of rest the nerve recovers its conductivity. The time required before even partial recovery takes place is unusually long ~~in one case over five minutes (fig. 5)~~, and a much longer period is required before the conductivity of the nerve is completely restored. (fig. 5) There is still a further point of difference. After the conductivity of the nerve for a low strength of stimulation (30 Kronecker units) has been abolished by repeated "tetanization", and before any recovery has taken place, the nerve always conducts at a higher intensity (100 Kronecker units).

The so-called "Wedensky effect" is not to be obtained with a protoveratrinised nerve . The nerve, it is true, conducts better with a slow rate of stimulation (25 per sec.) than with a rapid (100 per sec.) but with a

fixed rate and varying strength of stimulation there is no well-marked "optimum" height of tetanus, and further a series of strong excitations produces a better tetanus than a series of weak excitations. (fig. 3).

In the absence of the "Wedensky effect", and in the unusually long time required for recovery from fatigue, the results obtained from protoveratrinised nerves resemble very closely those obtained by Tait and Gunn from yohimbinised nerves.

D. Action of Aconitine.

It was to be expected from Waller's experiments that the action of aconitine on nerve fibres would be similar to that of protoveratrine. This was found to be the case when the aconitine was applied directly to the nerve.

When a solution of aconitine (the concentration used was 1 in 10,000) is thus applied, the tracings are ~~indistinguishable from~~ similar to those obtained under similar circumstances with protoveratrine (fig. 4). The nerve shows "fatigue" when subjected to repeated periods of stimulation; it recovers after a sufficient period of rest: and when, as a result of fatigue, it has become inexcitable to a low intensity of stimulation, it still responds to a higher intensity. ~~This~~ There is no "Wedensky effect."

The resemblance, however, ceases there. The tracings given by a nerve-muscle preparation taken from a frog that has been injected with aconitine are similar to

those obtained when aconitine is applied directly to the nerve (~~fig. ——~~). The permanent fatigue with the absence of response to an increase in the strength of the stimulus, which are the outstanding features when a preparation from a protoveratrinised frog is tested, were never seen in a preparation from a frog injected with aconitine.

E. The Site of the Permanent Fatigue.

It has been seen that with protoveratrine different results are obtained according as the alkaloid is injected into the animal or applied directly to the nerve. Four explanations of this difference are possible. (The action upon the muscle fibres is, as has been stated, negligible).

(1). In a preparation from an injected frog, the whole length of the nerve is exposed to the action of the protoveratrine. When the drug is applied directly, only a small portion of the nerve is affected. The divergence in the results might be due to the variation in the length of nerve affected by the protoveratrine.

(2). Protoveratrine may form some compound in the blood whose action differs from that of the alkaloid in simple solution.

(3). In the first series of experiments the portion of the nerve stimulated was itself impregnated with protoveratrine. This might affect the result.

(4). Protoveratrine may have a special action upon the myo-neural junction apart from its action upon

nerve fibres.

These suggestions will be discussed in order.

(1). Variations in the length of nerve exposed to the action of the drug do not affect the nature of the result. Preparations in which the protoveratrine solution was applied to practically the whole length of the dissected nerve gave similar tracings to those in which the alkaloid was applied to less than ¼ in. of the nerve.

(2). The suggestion that the protoveratrine might form some compound in the blodd whose action differed from that of the alkaloid itself was disproved in two ways :-

(1) Frogs were perfused through the aorta with a solution of protoveratrine after their blood-vessels had been thoroughly washed out with Ringer's fluid. The preparations taken from these perfused frogs showed the same irrecoverable fatigue as those taken from injected frogs.

(2) A frog was killed with a large dose of protoveratrine and the serum from its blood applied to the nerves of two nerve-muscle preparations. The effect was exactly similar to that obtained by the use of a solution of protoveratrine in saline.

(3). It was easily shown that the result was not affected by the portion of nerve stimulated having been previously exposed to the action of protoveratrine. Dissected nerve-muscle preparations were immersed in a bath of protoveratrine solution in such a way that the portion of the nerve to which the electrodes were applied was held above the level of the liquid. As soon as the muscular response began to fall off (which was in about three hours with the strength of solution used) the preparation was tested in the usual way. The tracings were similar to those obtained from preparations taken from injected frogs.

(4). There remains, then, only the last hypothesis, that protoveratrine has a special action upon the myoneural junction. This was proved in the following manner. The muscle and end-plates were protected by ligature of the thigh, as in Claude Bernard's curari experiment. The protoveratrine was then injected, and the animal killed after the usual time had elapsed. Both preparations were then dissected out. That from the limb which was not ligatured gave the usual tracing. The protected preparation, however, gave a tracing exactly similar to that obtained when protoveratrine is applied directly to the nerve, showing both the recovery after rest and the response to an increase in the

strength of the stimulus (~~fig. —~~).

We may therefore refer the irrecoverable nature of the fatigue and the absence of response to an increase in the strength of the stimulus, seen in preparations from injected animals in which the muscle and nerve endings have not been protected, to the action of protoveratrine upon the myo-neural junction. We may furthermore conclude that while aconitine has an action upon nerve fibres similar to that of protoveratrine, it is without the action upon the myo-neural junction which is so marked with the latter drug.

Blake Brown

This fatigue of the myo-neural junction cannot be brought about by reflex stimulation of the frog during the period of poisoning. Experiments were performed in which frogs were kept constantly stimulated by pinching the skin during the interval between injection and dissection. By the end of about half an hour the animals were completely exhausted, and reflex movements could no longer be elicited. After one hour the nerve-muscle preparations were tested in the usual manner. These were found to be excitable thorugh the nerve in every case, and showed no more fatigue than preparations taken from injected frogs that had not been fatigued before death, and from which reflex movements were still to be

obtained. Since, however, very few stimulations are sufficient to produce permanent fatigue in the myo-neural junction from a frog poisoned by protoveratrine, it is evident that the motor endings in the intact animal must be protected from over-stimulation by a break-down of the reflex arc due to a paralysis either of the sensory nerves and endings or of the spinal cord.

The rapid fatigue which is such a prominent symptom in a frog poisoned with protoveratrine cannot therefore be due, as Watts Eden was inclined to think was the case, to the action of protoveratrine upon the peripheral motor apparatus.

Protoveratrine and Yohimbine.

As has been stated, Tait pointed out the remarkable resemblance between the pharmacological action of protoveratrine and that of yohimbine. Waller showed, however, that when tested electrically the action of the two alkaloids on nerve fibres is not the same.

When protoveratrine is tested by the method of rapid rythmical stimulation the muscle tracings obtained resemble very closely those given by Tait and Gunn for yohimbine. In both there is no "Wedensky effect"; both show loss of conductivity after repeated stimulation with recovery upon rest; in both the length of time required for re-

recovery is very much longer than that required with the ordinary anaesthetics. The recovery from fatigue is even slower with protoveratrine than with yohimbine.

One differenee in the action of these two substances was observed. Let us suppose that the method of stimulation be this. The nerve is subjected to successive periods of stimulation each lasting the same time, say 2 seconds. Between these periods of stimulation intervals of rest are interpolated each of the same duration, say 2, 3 or 4 seconds as the case may be. Under these circumstances, with yohimbine narcosis of the nerve the successive muscular responses after the first are all of equal amount,- the exact amount of recovery in each case depending on the length of rest. With protoveratrine narcosis, on the other hand, the successive muscular responses become progressively less and less (fig. 3). In other words, the amount of recovery in a given time is not a function merely of the amount of the immediately preceding excitation, but rather of the amount of other periods of excitation as well. In order that each successive recovery after stimulation should be equal, progressively increasing periods of rest are necessary.

The action of protoveratrine upon nerve,

however, as shown by the muscular response, resembles that of yohimbine very much more closely than was to be expected from a study of the results obtained electrically by Waller. The correlation of the muscular and electrical responses still requires further work.

The Nature of the Fatigue.

It may be worth while pointing out that the fact of a nerve showing "fatigue" while under the influence of a drug is no proof that fatigue can occur in a normal nerve. A simulation of fatigue may conceivably occur in a drugged nerve whether the passage of the impulse involves consumption of energy or is a purely physical process.

Experiment shows that the phenomenon accompanying the passage of an impulse involves some upset of internal equilibrium, followed immediately by a restoration to the previous resting state. A number of theories have been devised to explain the process on a merely physical basis. For the sake of argument we shall take any one of the theories that presupposes some physico-chemical dissociation and reassociation in the nerve, and show how the introduction of an extraneous substance might so interfere with this process as to bring about a series of phenomena closely simulating fatigue. The same mode of

reasoning may be applied, mutatis mutandis, to any other physical theory.

Assume that in the upset of equilibrium accompanying the passage of an impulse some chemical molecules or entities are temporarily released from a position of restraint. The drug may unite more readily with the molecules when they are in this freed state than when they are built up in combination or otherwise held in a position of restraint. The union of these portions of the conducting substance with the drug interferes with the reconstructive process, on the completion of which the possibility of the passage of the next impulse depends. The essential point to keep clear in mind is that during the dissociation the drug may combine more readily with some necessary part of the conducting molecule than when this portion is built up into the position that it occupies in the resting condition of the nerve. In other words, what seems at first sight to be fatigue may be merely a more rapid poisoning due to the fact that activity of the nerve favours the combination of drug and nerve substance.

That the drug must be supposed to combine with the intact or non-dissociated molecule is evident from the fact that when the nerve is left soaking in a solution it ultimately loses its

conductivity. In the case of protoveratrine and yohimbine narcosis, soaking the poisoned nerve in saline solution does not restore conductivity. The inference is that those drugs form a fairly stable union with the nerve substance.

SUMMARY.

(1). The action of protoveratrine upon nerve fibres, when tested by the method of rapid serial stimulation, the muscle response being used as an index of the processes occurring in the nerve, closely resembles that of yohimbine. A protoveratrinised nerve shows "fatigue" upon activity and recovery after rest. A rest of several minutes is sometimes necessary before the conductivity of the nerve is restored. There is no "Wedensky effect."

(2). There are, however, certain differences between protoveratrine and yohimbine.

Protoveratrine produces a more lasting "fatigue" in nerve fibres than does yohimbine. Again, the snap reflex in the frog produced by yohimbine is not produced by protoveratrine.

(3). The action of aconitine upon nerve fibres is similar to that of protoveratrine.

(4). Protoveratrine has a special action upon the myo-neural junction. After this has been exposed to the action of protoveratrine, stimulation through the nerve causes a rapid and permanent "fatigue".

(5). Aconitine is without this action.

REFERENCES.

(1) Watt & Eden., Arch. f. Path. u. Phar., 1892, Vol. XXIX. Pp. 440.

(2) Waller., Quart. Journ. Exp. Physiol., Vol.III, P.96.

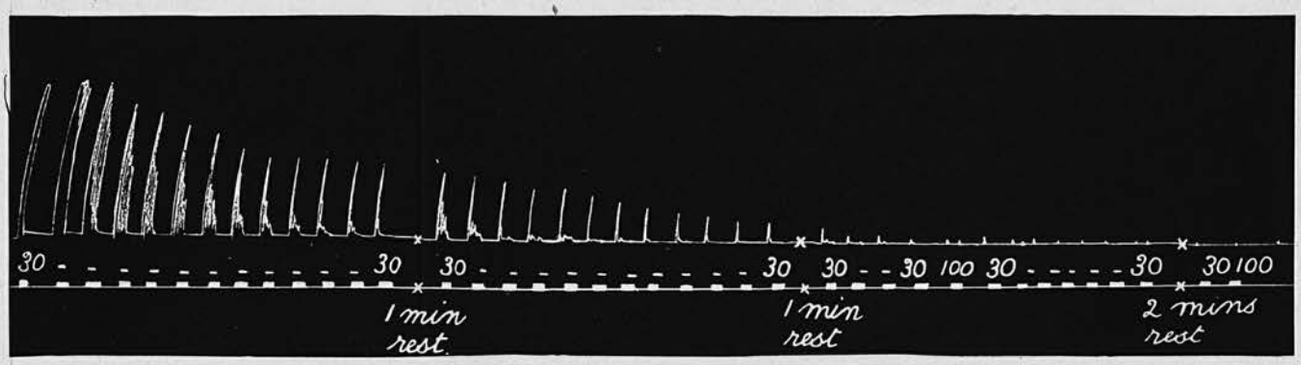
(3) Tait., Quart. Journ. Exp. Physiol., Vol.III, P.230.

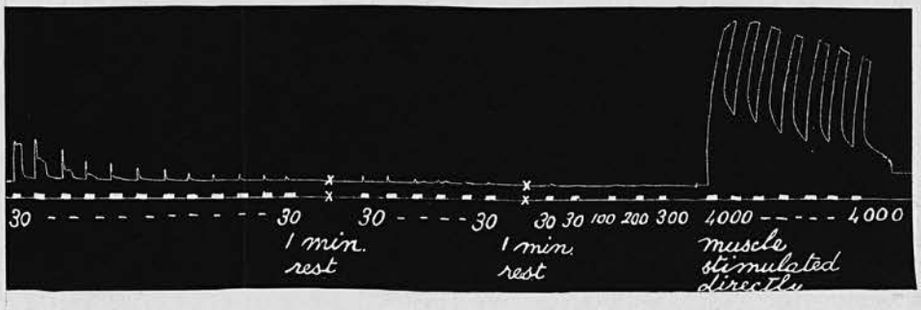
(4) Waller., Proc. Physiol. Soc., Nov., 1910., Journ. Phys. XLI.

(5) Wedensky., Arch. f. d. ges. Physiol. Bd. LXXXII, p. 134.

(6) Tait and Gunn., Quart. Journ. Exp. Physiol., Vol.I, p.191.

(7) Gunn., Quart. Journ. Exp. Physiol., 1908., Vol.I, p.111.





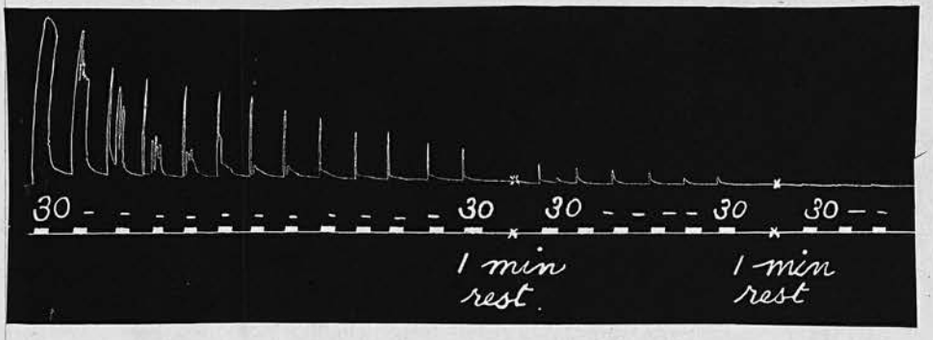


FIG. 1.—Three separate records of nerve-muscle preparation taken from frogs that had been injected 1 hour previously with protoveratrine—dose, ·02 mg. per gram weight of frog.

(Rate of drum, 60 mm. per minute; rate of stimulation, 100 per second. The duration of each period of stimulation and of the intervening rests is shown by the signal. Intensity of stimulation marked in Kronecker units, 2 volts in primary circuit of coil.)

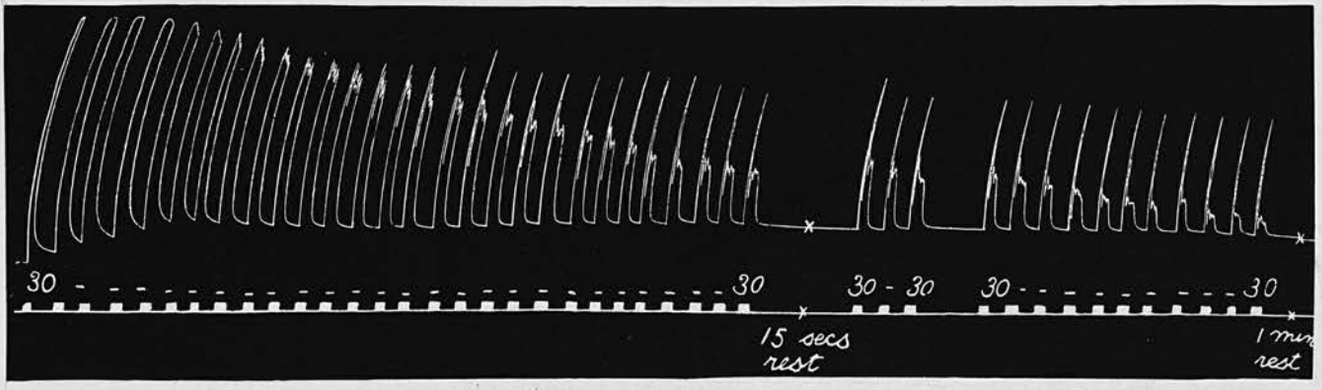
All the tracings show the rapid and permanent nature of the "fatigue."

(1) During inscription of the upper tracing the drum was stopped thrice, giving rest intervals respectively of 1 minute, 1 minute, and 2 minutes. After the first rest there is perhaps a slight recovery, after the others no recovery. Once the preparation has become inexcitable, a rise in intensity of stimulation from 30 to 100 Kronecker units produces no change in response.

(2) The middle tracing, of a preparation from a starved animal, shows more rapid "fatigue." As in (1) it can be seen that the degree of response of the fatigued preparation is independent of the intensity of stimulation. There is no recovery after rest intervals of 1 minute. The muscle responds to direct stimulation.

(3) The lowest tracing shows a falling off in response during rest.

Fig 2.



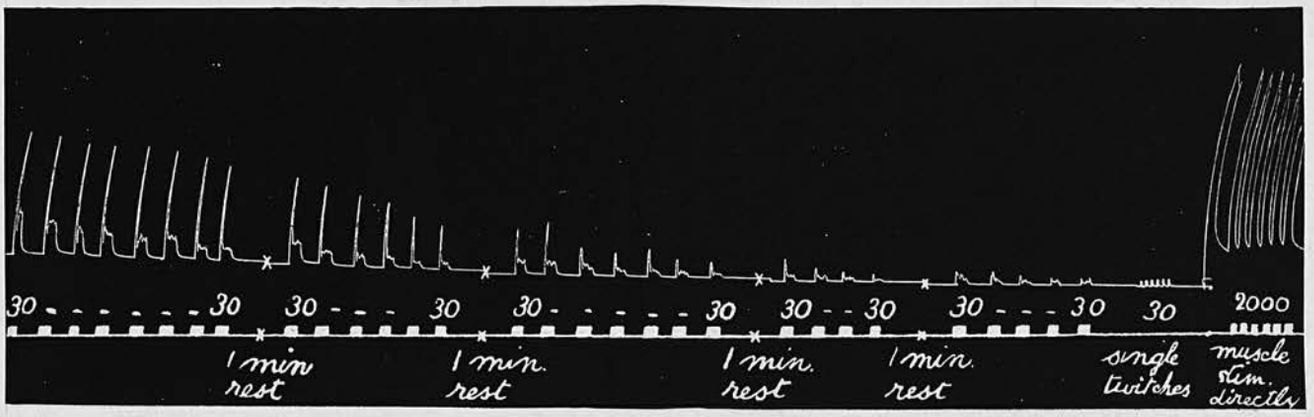


FIG. 2.—Record of a protoveratrinised nerve-muscle preparation showing a slighter degree of narcosis than in fig. 1. The tracing has been divided, and the lower record should be read continuously with the upper. The frog was injected 35 minutes before the preparation was dissected out.

(Rate of drum, rate of stimulation, dose, etc., as described in fig. 1.)

The tracing shows that in early protoveratrine narcosis a certain amount of recovery occurs after a relatively prolonged rest from stimulation. As the stimulation proceeds, however, i.e. towards the end of the tracing, recovery does not occur after equally long rests. The muscle is still excitable to direct stimulation after it has almost ceased to respond to indirect stimulation.

Fig 3.

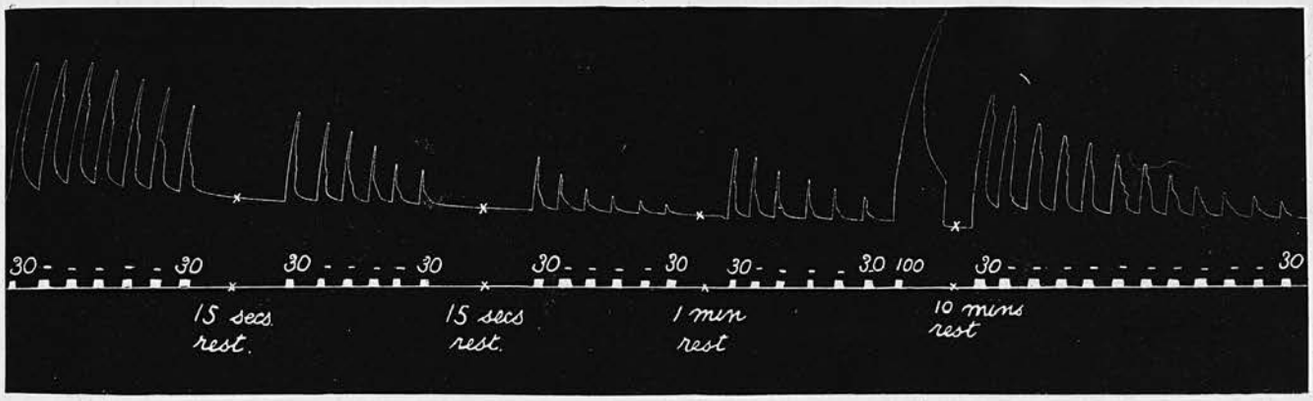


FIG. 3.—In this case the protoveratrine has been directly applied to the middle portion of the nerve of a fresh dissected preparation.

1·5 cm. of nerve was soaked for 2½ hours in blood serum from a 40-gram frog, into the dorsal lymph sac of which 5 mg. of protoveratrine had been injected 20 minutes before the blood was collected.

(Rate of drum, number of stimuli per second, etc., as described in fig. 1.)

In this tracing note :—

i. That the responses become progressively less and less when the intervals of rest are short, but with longer intervals of rest recovery occurs ; after a very long rest (10 minutes) recovery is perfect—the "fatigue," in other words, is not permanent.

ii. When the preparation has been temporarily fatigued by repeated stimulation at intensity 30 (see group 4), an increase in the intensity to 100 Kronecker units produces a strong and lasting tetanus of the muscle.

Similar tracings are obtained when a solution of protoveratrine in saline is applied to the nerve.

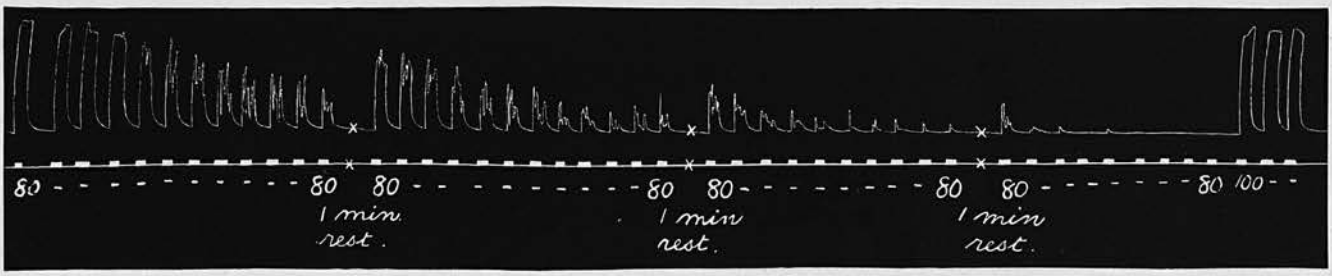


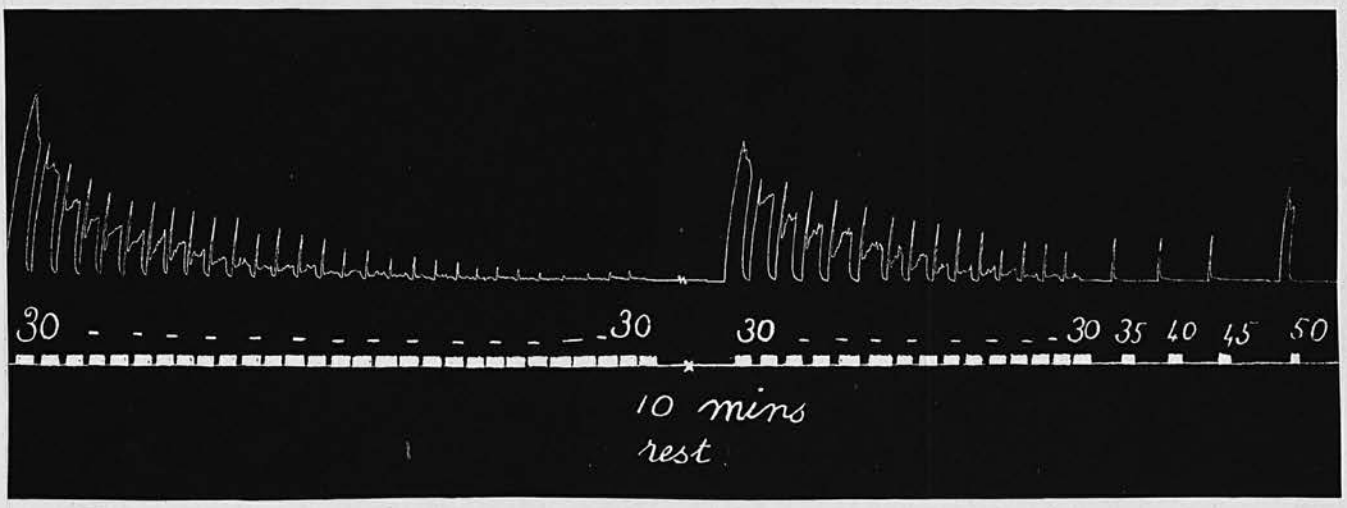
FIG. 4.—Record of nerve-muscle preparation taken from a 12-gram frog which had been injected 45 minutes previously with aconitine nitrate.
(Rate of drum, rate of stimulation, etc., as described in fig. 1.)

Note :—

i. That the responses become progressively less and less when the intervals of rest are short, but with longer intervals recovery occurs. Recovery, however, is never so complete in an aconitinised nerve as in a protoveratrinised nerve.

ii. When the preparation has been completely "fatigued" by repeated stimulation at intensity 80, an increase in the intensity of the stimulation to 100 Kronecker units results in a full tetanus of the muscle.

Similar tracings are obtained when aconitine is applied directly to the nerve, or when it is injected, the myo-neural junction being protected by ligature.



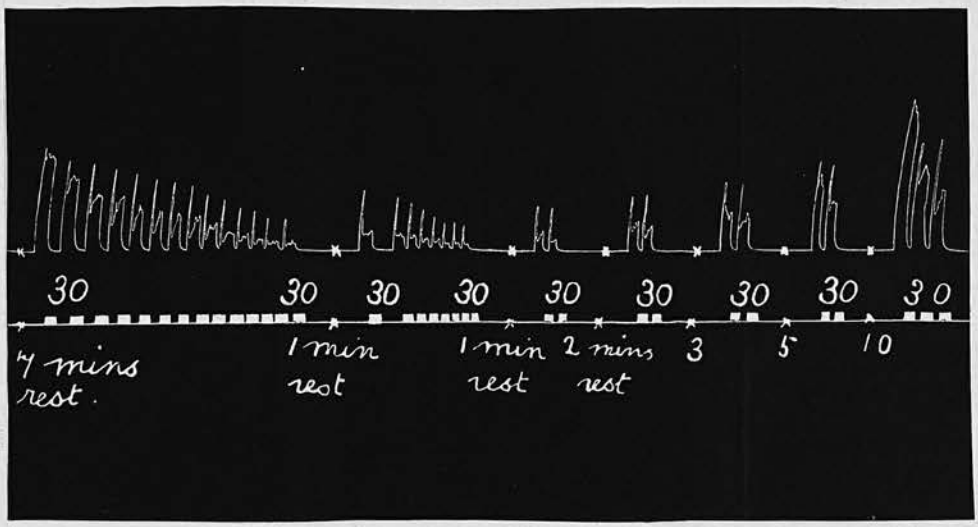


FIG. 5.—Record of a preparation the nerve of which has protoveratrinised by injecting the frog 1 hour previously, the myo-neural junction being meanwhile protected by a ligature. The tracing has been divided, and the lower record should be read continuously with the upper.

(Rate of drum, rate of stimulation, dose, etc., as described in fig. 1.)

Note:—

i. That with sufficient rest recovery may be complete even after the nerve has been many times fatigued, almost to loss of conductivity.

ii. That with successive longer and longer intervals of rest (see especially the lower part of the tracing) recovery is more complete.

iii. That when the responses have temporarily diminished by stimulation at intensity 30, an increase in the intensity of the stimulation produces an improvement in the muscle response (see the end of the upper portion of the tracing).